Interaction of Estramustine with Cytoskeletal Proteins and Alteration in their Regulation in Drug Resistant Prostate Carcinoma Cells. Lisa A. Speicher, Linda R. Barone, Naomi Laing, Joan D. Robbins,* Kenneth B. Seamon* and Kenneth D. Tew. Department of Pharmacology, Fox Chase Cancer Center, 7701 Burholme Avenue, Phila., PA and *Molecular Pharmacology Laboratory, Division of Biochemistry and Biophysics, Food and Drug Administration, Bethesda, MD.

Clonal selection of two human prostate carcinoma cells lines (E4 and E9) has resulted in stable expression of approximate 5-fold resistance to the antimicrotubule drug estramustine. Drug-induced antimitotic effects producing IC_{50} cytotoxicity occur at 12.5 M (resistant cells) compared to 2.5 M for the wild type DU145 cells. To identify specific drug targets, a photoaffinity analog, 17-O-[[2-[3-(4-azido-3-[^{125}I]iodophenyl)propionamido]ethyl]carbamyl]estradiol-3-N-bis(2-chloroethyl)carbamate (^{125}I AIPP-EM) was synthesized and reacted in competition assays with cytoskeletal protein preparations. Specific labelling of a 210 kD microtubule protein which immunoreacted with a polyclonal antibody to MAP-4 was found. Although the highest amount of labelled drug was associated with tubulin, excess unlabelled estramustine failed to prevent or compete this binding, suggesting non-specific or low avidity drug binding to the major microtubule protein. The E4 and E9 cells are approximately half the size of the wild type, but on a comparative basis, showed no difference in the protein levels of MAP-4, α or β-tubulin and β-actin. In contrast to protein expression, Northern blot analysis revealed that in E4 and E9 cells mRNA levels for MAP-4, β-tubulin and β-actin were elevated 10- to 20-fold when compared to the wild type. For α-tubulin, mRNA levels were similar. Since the altered transcript levels for β-tubulin and β-actin in the resistant cells were not accompanied by increased protein levels, studies to determine the half-lives of these proteins in both the wild-type and resistant cells were initiated using the ^{35}S-methionine pulse-chase technique. The resistant cells did not exhibit a shorter half-life for these proteins relative to the wild-type cells. Although the ratio of soluble versus polymerized tubulin is known to play a role in autoregulation of β-tubulin mRNA, no significant difference in this ratio was observed between the WT and EMR cells. However, our data are consistent with the principle that the MAP binding and antimicrotubule properties of estramustine can cause an imbalance in the transcripts coding for both major microtubule and microfilament proteins, suggesting that coordinate regulatory mechanisms may be affected.